# THE RESPIRATORY GAS EXCHANGE IN TERMOPSIS NEVADENSIS

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The series of studies made in recent years by the members of the Department of Zoölogy at the University of California, as well as other investigations, have thrown much light on the relations between environmental factors and the habits of termites, particularly with respect to diet, moisture, and symbiotic microörganisms. Questions have arisen at times in connection with other ecological problems pertaining to the utilization of and the dependence on oxygen by the termite colony. In particular, it is conceivable that small differences in the oxygen tension of various habitats might influence the distribution of species and life cycle of individuals. In considering the matter from the ecological point of view, one is confronted by the difficulty that very little is known about the normal respiration of the termite itself. It was to obtain information concerning this point that the present study was made.

Several hundred specimens of *Termopsis nevadensis,* secured from the vicinity of Santa Cruz, California, were kept in open dishes, and fed a combination of moist filter paper and wood scraps. For experimental work they were sorted roughly into sizes, use being made of only the nymphs. Although such is probably not the case, there is a possibility that the respiration of the soldiers and reproductives might differ from that of the nymphs. Both oxygen consumption and carbon dioxide production were measured manometrically by the Warburg method. Usually the gas exchange of about twelve termites at a time was measured in each manometer vessel. The results were sufficiently consistent to indicate that variations between individuals were thereby eliminated. In most cases the termites were weighed and the results expressed as cubic millimeters of oxygen absorbed or carbon dioxide evolved per gram per hour. Occasionally, however, this was not necessary, particularly when the results were purely comparative in nature.

It is, of course, very difficult to keep the termites motionless and otherwise achieve basal conditions. This must be constantly borne in mind in determining respiratory quotients. Cleveland (1925) has reported a quotient of 1.0, but since he does not state the conditions under which the measurements were made, it is to be assumed that he was dealing with normal, active animals, feeding on a preponderantly carbohydrate diet, animals which would be expected to show such a quotient. However, in investigating the respiration of invertebrates, both anaërobic and aërobic, it is not necessary to achieve that particular basal state demanded in a mammal, provided the conditions are uniform throughout the entire series of experiments. With termites these conditions may be attained satisfactorily, as is shown by the following experiment.

*Experiment 1.*—The respiration of ten groups of termites with varying number of individuals in each was measured at 32° C. The oxygen consumption in cubic millimeters per gram per hour was: 930, 820, 850, 740, 740, 830, 760, 750, 770, 860. The extreme variation is about 20 per cent.

The possibility should not be overlooked, in dealing with large numbers of termites of somewhat different ages, that the respiratory rate may vary with age. The possibility is eliminated, however, as indicated thus:

*Experiment 2.*—The oxygen consumption of two groups of termites was measured at 20° C., the first group consisting entirely of individuals of an average length of 10 millimeters, the second of 15 millimeters. The consumption of Group I was 433 cu. mm. $O_2$/gram $\times$ hour and that of Group II was 413 cu. mm. $O_2$/gram $\times$ hour, the difference lying within the experimental error.

The general problem of the relation between oxygen tension and oxygen consumption has been the subject of numerous investigations. Without entering at this point into any detailed discussion of the results of this work, it may be stated that there has not yet been found any clear correlation between the two. Some animals are able to acquire and use their normal amount of oxygen at extremely low tensions; others are very closely dependent upon the tension. The characteristic reaction of each species must be determined experimentally in every case. (For reviews of the literature, reference may be made to papers by Helff, 1928, and Hyman, 1929.)

*Experiment 3.*—To ascertain the general relation between oxygen tension and consumption a series of comparative measurements were made with several groups of animals at 20° C., using oxygen concentrations of 100, 21, 10, 5, 2, and 0.8 per cent. For the lowest tension a tank of commercial nitrogen served with oxygen present to the extent of 0.8 per cent as an impurity. The procedure in all cases was to establish the normal rate in air and then to replace the air in the manometer vessel with the gas mixture to be investigated. Frequently two or more mixtures were introduced successively and when this was done the order was varied from group to group. Check readings were made at the end in air to insure that no injury has been done the respiratory mechanism through subjection to low or high oxygen tensions. No abnormalities were observed save in the case of 0.8 per cent. The rate in air after exposure to this tension was a little low but soon rose to the normal. The initial low rate was probably due to the immobility of the animals which is induced by very low tensions. To the immobility

TABLE I

*Relative Oxygen Absorption of Termopsis at Varying Oxygen Tensions*

| Group no. | Oxygen concentration | Oxygen absorption |
|---|---|---|
| | *per cent* | *per cent of normal* |
| I | 100 | 111 |
| II | 100 | 93 |
| III | 100 | 86 |
| IV | 100 | 82 |
| I | 21 | 100 |
| II | 21 | 100 |
| III | 21 | 100 |
| IV | 21 | 100 |
| V | 21 | 100 |
| VI | 21 | 100 |
| I | 10 | 88 |
| II | 10 | 93 |
| I | 5 | 86 |
| II | 5 | 83 |
| III | 2 | 64 |
| IV | 2 | 73 |
| V | 0.8 | 26 |
| VI | 0.8 | 30 |

may also be partially ascribed the low respiratory rate *during* exposure to 0.8 per cent oxygen. Since all these rates are relative and are compared to the rate in air as normal, they may be expressed on a percentage basis, taking the rate in air as 100 per cent. The data are summarized in Table I. It will be noted that as the tension decreases, the relative oxygen consumption also decreases, but not proportionally.[1] The respi-

[1] The effect of pure oxygen appears to be slightly inhibitory. This phenomenon has been observed in the case of other organisms but has never been satisfactorily explained. A suggestion which might be advanced is that the oxygen at high tension inhibits the respiration of the microörganisms of the gut. This fauna is killed by prolonged exposure to oxygen and may be sensitive to shorter exposures.

ration remains very near the normal until tensions below 5 per cent are reached and even with less than 1 per cent oxygen the respiration is almost one-third of its usual value. This situation indicates a very high degree of independence of the oxygen tension on the part of the termite. Furthermore, there appears to be a very well-developed capacity for utilizing extremely small quantities of oxygen. This capacity was made evident in the above experiment when the gas used was 0.8 per cent oxygen in nitrogen. For here the rate of respiration underwent a steady decrease, indicating that the oxygen tension was being materially reduced by the termites themselves and that the rate decreased along with the tension.

It seemed advisable to investigate more thoroughly this ability of the termites to utilize very small concentrations of oxygen. Therefore a series of studies was made, the data for which are consolidated in Table II. For the purpose of orientation a group of normal termites was first used with a mixture containing 0.7 per cent oxygen and the gas exchange followed for several hours (data not shown in Table II). The oxygen uptake was steady for a short time (about 30–40 minutes), then began to fall off. The decrease continued until the gas consumption ceased. But on continuing the readings it was observed that a positive pressure appeared, suggesting that now some other gas was being evolved. The rate of evolution became constant within an hour and remained so as long as measurements were continued—a matter of several hours. Since the vessel contained strong potassium hydroxide, this gas could not be carbon dioxide. The experiment was repeated with an inset of 10 per cent sulphuric acid as well as alkali, but the general course of the reaction was similar. The termites therefore evolve a gas which can be absorbed by neither acid nor alkali. No further attempt was made to determine the exact composition of this substance, but there is a strong possibility that it may be hydrogen or methane, or a mixture of both. If so, a reasonable assumption is that the microorganisms in the gut are responsible. The principal constituent of the termite diet is cellulose and the breakdown of this material is usually ascribed to the protozoa and possibly bacteria which inhabit the digestive tract. In other animals which utilize cellulose in a similar manner, such as cattle, large amounts of hydrogen and methane are produced. There is therefore considerable likelihood that we are dealing with an analogous situation in the termite, although naturally such a statement cannot be made with certainty in the absence of a quantitative analysis of the gas produced.

In order to investigate the rôle of the intestinal fauna in the production of this material—which we may call for lack of a more exact description the "undetermined" gas—a number of termites were de-

TABLE II

*Oxygen Absorption by Termites at Very Low Tensions*

| A. Group | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|
| B. Previous treatment of termites | Normal | Normal | Normal | Defaunated | Defaunated | Defaunated | Defaunated |
| C. Initial $O_2$, per cent | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 2.08 | 2.08 |
| D. Weight in mg | 513 | 1067 | 457 | 552 | 1126 | 386 | 699 |
| E. Duration of experiment in hours | 6 | $6\frac{5}{6}$ | $7\frac{3}{4}$ | $6\frac{1}{2}$ | 6 | $10\frac{1}{2}$ | $6\frac{1}{2}$ |
| F. Net gas exchange in cu. mm. | −0.5 | +52 | −4.5 | −83 | −88 | −333 | −271 |
| G. Rate of production of undetermined gas in cu. mm. per hour | 11.3 | 19 | 9 | 0 | 0 | 0 | 0 |
| H. Total production of undetermined gas in cu. mm. | 79 | 130 | 77.5 | 0 | 0 | 0 | 0 |
| I. Rate of production of undetermined gas in cu. mm. per gram termite per hour | 22.1 | 17.8 | 19.7 | 0 | 0 | 0 | 0 |
| J. Total gas in manometer vessel in cu. mm. | 13,150 | 12,650 | 13,200 | 13,110 | 12,450 | 15,250 | 12,850 |
| K. Oxygen at beginning of experiment in vessel in cu. mm. J × C | 92 | 88 | 92 | 92 | 87 | 318 | 270 |
| L. Total oxygen absorbed in cu. mm. F − H | 79.5 | 78 | 74.5 | 83 | 88 | 333 | 271 |

faunated. This was done by the method of Andrew (1930) which consists of the application of oxygen at several atmospheres pressure. When defaunated termites are placed in 0.7 per cent oxygen there is no indication whatever that any gas is evolved. The oxygen consumption sinks to zero and remains there indefinitely. It seems legitimate therefore to ascribe the inert gas production to the intestinal fauna.

The complicating factor of formation of the undetermined gas may thus be eliminated by defaunation, but the question is introduced whether defaunated and normal termites react in the same way to low oxygen tensions. To investigate this matter and simultaneously to secure data concerning the original problem of utilization of small amounts of oxygen the following experiment was performed.

*Experiment 4.*—Seven groups of termites were placed in low concentrations of oxygen. Groups I, II, and III were normal, the remainder were defaunated. The first three groups and also Groups IV and V were placed in 0.7 per cent oxygen and Groups VI and VII in 2.08 per cent oxygen. The oxygen content of these mixtures was checked carefully by analysis with the Haldane gas apparatus. Then the gas exchange in each group was measured until, in the case of the first three groups, the rate of production of the undetermined gas was clearly established, and in the case of the others the oxygen consumption had ceased for at least two hours. At the end of this time the readings were discontinued and the *net gas exchange* of each group calculated. This, of course, is obtained from the initial and final manometer readings (see line F in Table II). With the defaunated groups the net exchange is equal to the total oxygen absorption, since there is no other gas concerned. With the normals the net exchange is equal to the oxygen absorbed plus the undetermined gas evolved. To find the total oxygen absorption we must subtract from the net exchange the total undetermined gas evolution. This involves the fairly reasonable assumption that the production of undetermined gas has the same constant value while oxygen is being taken up that it is observed to have after oxygen uptake ceases. This assumption, though reasonable, still remains an assumption, for definite evidence cannot be secured until a method is devised for differentially absorbing the undetermined gas in the vessel as fast as it is formed. There appears to be no method at present for doing this. If we, then, subtract the total undetermined gas evolved from the net gas exchange, we get the probable total oxygen consumption of the normal groups (see line L in Table II). Finally, since the volume of the vessels is known and also the percentage composition of the gas initially introduced, the actual initial quantity of oxygen can be calculated (see line K in Table II). The total oxygen usage may then be compared with the total oxygen available.

From the data presented in Table II the following conclusions may be drawn. In the defaunated groups (IV–VII inclusive) there is a fairly close correspondence between the oxygen absorbed and the amount initially present in the closed system. At least it may be stated that there is no significant quantity of oxygen remaining in the system when the oxygen consumption of the termites ceases. This appears to be true irrespective of the initial concentration (compare Groups IV and V with VI and VII). In the normal termites there seems to be a slight residue of oxygen, a concentration of the order of 0.1 per cent or a tension of less than one millimeter of mercury. But this difference between the normal and defaunated animals is too slight to be of significance, particularly since (1) the difference is of the order of the experimental error (as indicated by the deviations in Groups IV–VII) and (2) the assumption of a constant rate of inert gas evolution, irrespective of oxygen tension, may not be precisely consistent with the facts. In general, however, it is possible to state that both normal and defaunated termites are able to utilize substantially all the oxygen in the immediate environment even though the latter reaches exceedingly low tensions.

Under anaërobic conditions at least the production of undetermined gas is very constant (see line I in Table II) at a rate of about twenty cubic millimeters per gram termite per hour. A further study of this gas production would be of interest with respect to the composition of the gas and also its possible bearing on the problem of cellulose digestion.

The results obtained with low oxygen tensions suggest further questions: (1) What is the aërobic carbon dioxide production in normal and defaunated animals? This involves also the determination of the respiratory quotient of both types of animal. (2) Is carbon dioxide produced anaërobically, and if so, can the termites incur an oxygen debt? (Such has been found to be the case with the cockroach by Davis and Slater, 1926, 1928.)

*Experiment 6.*—Eight groups of termites were investigated (see Table III). Groups I–IV were normal animals. Groups V–VII were defaunated one day previously and Group VIII was defaunated two weeks previously. In every case the gas exchange was determined in air in the presence of 5 per cent KOH (line C, Table III) and then in air without alkali (line D, Table III). When no gases other than oxygen and carbon dioxide are involved the result of the first determination represents the oxygen consumption, since the carbon dioxide is quantitatively absorbed by the KOH. The observed gas exchange in the second determination then represents the difference between the oxygen consumption and carbon dioxide production. Since the former is known, the latter may be calculated (line E, Table III). It was ascertained in the previous experiment (Experiment 5) that there is no production of the undetermined gas by defaunated termites and therefore in Groups V–VIII the observed exchange of carbon dioxide and oxygen may be taken as the corrected, or true, exchange (lines H and I, Table III). With normal termites it may be assumed, as previously, that

### Table III

*Carbon dioxide production and respiratory quotient of termites.* Gas exchange in all cases expressed as cubic millimeters per gram termites per hour.

| A. Group and condition | I Normal | II Normal | III Normal | IV Normal | V Defaunated 1 day | VI Defaunated 1 day | VII Defaunated 1 day | VIII Defaunated 2 wks. |
|---|---|---|---|---|---|---|---|---|
| B. Weight in mg. | 731 | 633 | 681 | 866 | 852 | 648 | 977 | 694 |
| C. Observed oxygen consumption in air (KOH) | 186 | 218 | 173 | 239 | 190 | 239 | 154 | 200 |
| D. Observed gas exchange in air (no KOH) | 11 | 14 | 21.5 | 22.5 | −3.5 | 2.0 | 4.0 | −36 |
| E. Observed carbon dioxide production (C–D) | 192 | 232 | 194.5 | 261.5 | 186.5 | 241 | 158 | 164 |
| F. Observed R.Q. | 1.06 | 1.065 | 1.12 | 1.095 | 0.98 | 1.005 | 1.025 | 0.82 |
| G. Production of undetermined gas (average from Table II) | 20 | 20 | 20 | 20 | 0 | 0 | 0 | 0 |
| H. Corrected oxygen consumption in air (KOH) C–G | 206 | 238 | 193 | 259 | 190 | 239 | 154 | 200 |
| I. Corrected carbon dioxide production in air (no KOH) C–D | 197 | 232 | 194.5 | 261.5 | 186.5 | 241 | 158 | 164 |
| J. Corrected R.Q. | 0.955 | 0.975 | 1.005 | 1.01 | 0.98 | 1.005 | 1.025 | 0.82 |
| K. Total gas production in nitrogen | 46.5 | 52 | 48.5 | 48 | 50.5 | 55.5 | 43 | 43.5 |
| L. Carbon dioxide production in nitrogen (K–G) | 26.5 | 32 | 28.5 | 28 | 50.5 | 55.5 | 43 | 43.5 |

under aërobic conditions there is a constant production of about twenty cubic millimeters of the undetermined gas per gram termite per hour; otherwise the respiratory quotient of the normal animals appears to be considerably over unity (line F, Table III). This factor may be allowed for and corrected values of the oxygen and carbon dioxide exchange obtained (lines H and I in Table III). Applying this correction, the values of the respiratory quotient approach very closely to unity (line J, Table III). The freshly defaunated termites also show the same approximate value, and with them no assumption concerning the production of the undetermined gas is necessary. These results confirm the statement of Cleveland that the usual R.Q. of termites is practically 1.0. It is of interest to note, however, that termites which have been defaunated for some time and consequently probably are undergoing starvation show a much lower R.Q.—in the one case here investigated 0.82.[2] This is to be expected from what we know in general of the effect of starvation on the R.Q. of other animals.

To determine the anaërobic carbon dioxide production the same termites were subjected to as low an oxygen tension as could readily be obtained, 0.7 per cent. Since it was ascertained in Experiment 5 that the consumption of this oxygen ceases or is too small to have material effect in about three hours, the termites were allowed to remain for this period in the closed vessels and then the gas exchange was measured for one hour. The readings showed a low but distinct positive variation, indicating the production of gas (line K, Table III). With normal animals part of the output (20 cu. mm./gm. $\times$ hr.) was due to production of the undetermined gas and therefore this amount was subtracted from the observed gas production to give the carbon dioxide value (line L, Table III). This value is considerably below that obtained under aërobic conditions but is evidence that some metabolic changes are still proceeding in the animal's tissues.

To summarize this experiment and answer question 1, it may be said that both normal and freshly defaunated termites produce carbon dioxide under aërobic and anaërobic conditions; that the R.Q. of both types of animal is very close to unity; and that the anaërobic carbon dioxide production while present is much smaller in both types than the aërobic production.

*Experiment 7.*—Three groups containing equal numbers of termites at 20° C. were placed in air and their respiration measured with and without the presence of KOH. Group I was then exposed to 0.7 per cent oxygen in nitrogen in the presence of KOH for an hour and a half, or until the oxygen was nearly exhausted, at which time air was reintroduced. The oxygen consumption began again and after a brief interval at a lower rate proceeded indefinitely at the same rate as at the beginning.

[2] All the termites were normally mobile and active, even those which had been starved. All were therefore at the same "basal" level.

The absence of any increase in the rate above the normal precludes the possibility of oxygen debt. Groups II and III were similarly treated except that KOH was not present. Air was readmitted at the end of three hours of anaërobiosis. The respiration rates (both carbon dioxide and oxygen) returned very quickly to their normal values and remained there for several hours, at the end of which time the experiment was discontinued.

*Experiment 8.*—A repetition of Experiment 7 in which the results of the latter were confirmed.

It seems clear that the termite (*Termopsis* at any rate) possesses a mechanism for the continued production of carbon dioxide even in

TABLE IV

*The Effect of Prolonged Exposure to Nitrogen*

| Termite group | Normal oxygen consumption | Hours exposure to nitrogen | Oxygen consumption after exposure | Condition after recovery |
|---|---|---|---|---|
| | *per cent* | | *per cent of normal* | |
| I | 100 | 1 | 89 | Immediate recovery from immobility |
| II | 100 | 3 | 99 | Same |
| III | 100 | 6 | 86 | Recovery in 15 minutes |
| IV | 100 | 9 | 95 | Recovery in 20 to 30 minutes |
| V | 100 | 24 | 99 | Recovery in 1 hour |
| VI | 100 | 48 | 96 | Recovery in 12 hours |
| VII | 100 | 96 | 42 | Never recovered |
| VIII | 100 | 144 | 16 | Never recovered |

the complete absence of oxygen. That this mechanism is not identical with that which presumably obtains in mammalian muscle and elsewhere is demonstrated by the complete lack of any indication, from gas measurements at least, of oxygen debt. That the anaërobic system is rather inefficient is shown by the fact that the carbon dioxide production under such conditions is less than one-half the normal value and furthermore by the fact that the termites pass into a state of complete immobility even though they continue to respire. This state resembles acute anoxemia in mammals in that it appears very soon after a sudden exclusion of oxygen and disappears very quickly after readmission of oxygen, a matter of minutes in both cases.

It is worth while to determine how long *Termopsis* can endure complete anoxemia and still retain its capacity for normal aërobic respiration and normal activity.

*Experiment 9.*—Eight groups of termites, after their normal oxygen consumption was measured, were placed in a desiccator. The latter was filled with nitrogen from which the oxygen had been removed by treatment with strongly alkaline pyrogallol. At intervals the termites were removed and their respiration measured as well as their general behavior observed. The respiratory rate was measured after the termites had recovered their mobility, except in Groups VII and VIII which never recovered. The lack of mobility may therefore in part account for the low rate of oxygen consumption noted in these two groups. The effect of prolonged exposure to pure nitrogen is summarized in Table IV.

It will be observed that the first noteworthy reduction in respiration occurs in the group which had been without oxygen for four days and that this group never recovered their mobility. These termites therefore are not truly anaërobic in the same sense as, for example, yeast, which is able to survive and grow indefinitely in the absence of oxygen. Nevertheless the survival without apparent harm for two days is in itself a striking and significant phenomenon.

In addition to the ability to withstand oxygen lack, *Termopsis* shows high tolerance to the presence of carbon dioxide. The respiration of four groups of termites was measured in 20 per cent oxygen plus 5, 10, 20, and 40 per cent carbon dioxide respectively. The net gas exchange of those in the 5, 10, and 20 per cent carbon dioxide, with no alkali present in the vessels, was approximately the same as that of termites in air and the appearance of the termites otherwise was perfectly normal. It is unlikely therefore that any considerable upset occurred in their respiration. The same considerations concerning respiration apply to the group in 40 per cent carbon dioxide, but these termites soon became immobile.

The general conclusion may be drawn from all these experiments that *Termopsis nevadensis* possesses powers for meeting adverse environmental conditions far in excess of its probable needs. The natural habitat is relatively well aërated wood in which the gas tensions are probably not far from atmospheric. Nevertheless, these animals are capable of extracting practically the last traces of oxygen in a closed space and then of persisting several hours, if not days, in the absence of oxygen. Furthermore, they can endure quantities of carbon dioxide which would seldom if ever be present naturally in their environment. These conditions might occur in wet soil or in other habitats frequented by some of the other species and genera of termites. If this is so, it is possible that ability to withstand such conditions is a general characteristic of all the members of the group which persists in certain species even though the actual need is seldom encountered. A comparative study of the different species of termites with respect to their respiratory power and their environment is very desirable.

## Summary

The oxygen consumption of *Termopsis nevadensis* does not decrease materially with falling oxygen tension until a concentration of approximately two per cent is reached. Below this tension the affinity of the animals for oxygen is so marked that substantially all the available gas is consumed.

In the absence of oxygen the organism is able to respire anaërobically, although at a reduced rate, for as long as two days without injury. During this time the animals are in a state of immobility from which they recover soon after readmission of air. After exposure to anaërobic conditions no indication of oxygen debt was found.

These termites are able to exist and respire normally in carbon dioxide as high as 20 per cent. Higher concentrations tend to induce a condition of anæsthesia, which, however, is reversible.

Under anaërobic conditions, and possibly also in the presence of oxygen, the termites evolve an undetermined gas which may be hydrogen or methane. The production of this gas depends on the integrity of the intestinal fauna, since it is not evolved by defaunated termites.

The respiratory quotient of both normal and freshly defaunated termites is approximately unity, but in starved defaunated termites it falls considerably.

## LITERATURE CITED

Andrew, B. J., 1930. Method and Rate of Protozoan Refaunation in the Termite Termopsis angusticollis Hagen. *Univ. Calif. Publ. Zoöl.*, **33**: 449.

Cleveland, L. R., 1925. The Ability of Termites to Live Perhaps Indefinitely on a Diet of Pure Cellulose. *Biol. Bull.*, **48**: 289.

Davis, J. G., and W. K. Slater, 1926. The Aërobic and Anaërobic Metabolism of the Common Cockroach (Periplaneta orientalis). Part I. *Biochem. Jour.*, **20**: 1167.

Davis, J. G., and W. K. Slater, 1928. The Aërobic and Anaërobic Metabolism of the Common Cockroach (Periplaneta orientalis). Part III. *Biochem. Jour.*, **22**: 331.

Helff, O. M., 1929. The Respiratory Regulation of the Crayfish, Cambarus immunis (Hagen). *Physiol. Zoöl.*, **1**: 76.

Hyman, L. H., 1929. The Effect of Oxygen Tension on Oxygen Consumption in Planaria and Some Echinoderms. *Physiol. Zoöl.*, **2**: 505.